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Date of Signature and Deposit: 3-22, 2004


Sara D. Vinarov

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Frederick R. Blattner
Rodney A. Welch
Valerie D. Burland

Date: 3-22, 2004

Serial No.: 10/085,959

Group Art Unit:

Filed: 03/01/2002

Examiner:

Title: NOVEL SEQUENCES OF E. COLI CFT073

File No.: 960296.97648

**PETITION FOR CORRECTION OF FILING DATE
UNDER 37 C.F.R. 1.10**

Commissioner for Patents
Attention: Office of Patent Legal Administration
P O Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants hereby respectfully petition for correction of the filing date from "March 1, 2002" to --October 19, 2001-- based on the following remarks and evidence submitted herewith.

Remarks

The error was discovered on the original and the updated official filing receipts (copies attached). The return postcard (copy attached) shows the same error on the bar code label.

As evidence of the October 19, 2001 filing date applicants submit herewith the following:

- 1) A true copy of the express mail filing receipt from the U.S. Postal Service. The Express Mail label shows that the subject non-provisional patent application papers were deposited by Express Mail on October 19, 2001 at 4:58 p.m. The acceptance of the package was initialed by the U.S. Postal Service clerk.

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- 2) The patent application papers necessary for receiving a filing date, including items a) through d) having Express Mail No. ET943407105US:
 - a) a utility transmittal sheet,
 - b) a fee sheet,
 - c) a specification (including at least 1 claims),
 - d) "Sequence Listing Statement" attached to the specification, and
 - e) fully executed Declaration (timely filed in response to a Notice of Missing Parts on July 5, 2002).
- 3) The U.S. PTO log book page for October 19, 2001 from the Madison Office of Quarles & Brady LLP indicating that the U.S. patent application was filed on that date.

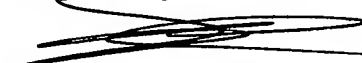
Notwithstanding the proper procedures followed by applicants, the subject application still received a filing date as of the date it was received in the U.S. PTO, which was March 1, 2002, nearly five months after the "Express Mail" filing date.

Applicants have been advised that presumably due to the anthrax problem, quite a bit of mail directed to the U.S. PTO during the last two weeks of October, 2001, was delayed in the U.S. Postal Office in Washington D.C.

Therefore, based on this evidence, it is respectfully requested that the filing date be corrected to October 19, 2001 and that a new filing receipt be issued.

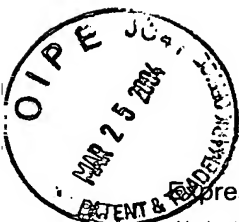
This Petition is accompanied by the petition fee (37 CFR 1.17(h)). However, it is not believed that any fee is due for this petition. In the event that the Office determines that a fee is due, the Commissioner is hereby authorized to charge any fee or credit any overpayment to Quarles & Brady LLP deposit account 17-0055.

Respectfully submitted,



Sara D. Vinarov
Reg. No.: 48,524
Attorney for Applicants
QUARLES & BRADY LLP
P.O. Box 2113
Madison, WI 53701

TEL 608/251-5000
FAX 608/251-9166



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Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$) 130.00

Complete if Known

Application Number	10/085,959
Filing Date	10/19/2001
First Named Inventor	Frederick R. Blattner
Examiner Name	
Art Unit	1632
Attorney Docket No.	960296.97648

METHOD OF PAYMENT (check all that apply)☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None☒ Deposit Account:Deposit Account Number: 17-0055
Deposit Account Name: Quarles & Brady LLP

The Director is authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☒ Credit any overpayments☒ Charge any additional fee(s) or any underpayment of fee(s)☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.**FEE CALCULATION****1. BASIC FILING FEE**

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1001 770	2001 385	Utility filing fee	
1002 340	2002 170	Design filing fee	
1003 530	2003 265	Plant filing fee	
1004 770	2004 385	Reissue filing fee	
1005 160	2005 80	Provisional filing fee	
SUBTOTAL (1)			(\$) 0.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Extra Claims	Fee from below	Fee Paid
Independent Claims	-20* =	X	0.00
Multiple Dependent	-3** =	X	0.00

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description
1202 18	2202 9	Claims in excess of 20
1201 86	2201 43	Independent claims in excess of 3
1203 290	2203 145	Multiple dependent claim, if not paid
1204 86	2204 43	** Reissue independent claims over original patent
1205 18	2205 9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$) 0.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)**3. ADDITIONAL FEES**

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1051 130	2051 65	Surcharge - late filing fee or oath	
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130	Non-English specification	
1812 2,520	1812 2,520	For filing a request for <i>ex parte</i> reexamination	
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action	
1251 110	2251 55	Extension for reply within first month	
1252 420	2252 210	Extension for reply within second month	
1253 950	2253 475	Extension for reply within third month	
1254 1,480	2254 740	Extension for reply within fourth month	
1255 2,010	2255 1,005	Extension for reply within fifth month	
1401 330	2401 165	Notice of Appeal	
1402 330	2402 165	Filing a brief in support of an appeal	
1403 290	2403 145	Request for oral hearing	
1451 1,510	1451 1,510	Petition to institute a public use proceeding	
1452 110	2452 55	Petition to revive - unavoidable	
1453 1,330	2453 665	Petition to revive - unintentional	
1501 1,330	2501 665	Utility issue fee (or reissue)	
1502 480	2502 240	Design issue fee	
1503 640	2503 320	Plant issue fee	
1460 130	1460 130	Petitions to the Commissioner	130
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)	
1806 180	1806 180	Submission of Information Disclosure Stmt	
8021 40	8021 40	Recording each patent assignment per property (times number of properties)	
1809 770	2809 385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810 770	2810 385	For each additional invention to be examined (37 CFR 1.129(b))	
1801 770	2801 385	Request for Continued Examination (RCE)	
1802 900	1802 900	Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$) 130.00

SUBMITTED BY

Name (Print/Type) Sara D. Vinagov

Registration No. 48,524
(Attorney/Agent)

(Complete if applicable)

Telephone 608/251-5000

Signature

Date 3-22-2004

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

373465

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Blattner, et al.
For: NOVEL SEQUENCES OF E.COLI CFT073

Date: October 19, 2001
Docket: 960296.97648

Commissioner for Patents:

Please acknowledge receipt by applying the Patent and Trademark Office receipt stamp and the serial number hereto and returning the same to me.

Express Mail Letter (No. ET943407105US)

Utility Patent Application Transmittal

Fee Transmittal (2 copies)

Application, 6 pages of specs

2 pages of claims (11 claims)

1 page abstract

0 pages of drawings

756 pages sequence listing

~~Declaration and Power of Attorney~~

~~Diskette Containing Sequence Listing~~

~~Statement Under 37 C.F.R. 1.821(f)~~

JC986 U.S. PTO
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03/01/02

[Signature]
Bennett J. Berson, 37,094



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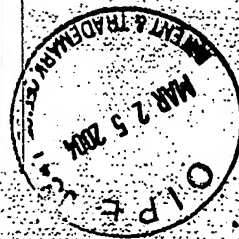
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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
10/085,959	03/01/2002	1632	908	960296.97648		11	5

CONFIRMATION NO. 4213

FILING RECEIPT



OC000000007794683

Nicholas J. Seay
One South Pinckney Street, Suite 600
P.O. Box 2113
Madison, WI 53701-2113

Date Mailed: 04/04/2002

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).

Applicant(s)

Frederick R. Blattner, Madison, WI;
Rodney A. Welch, Madison, WI;
Valerie D. Burland, Cross Plains, WI;

Domestic Priority data as claimed by applicant

Foreign Applications

If Required, Foreign Filing License Granted 04/04/2002

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

Title

Novel sequences of E. coli CFT073

Preliminary Class

435

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Title 37, Code of Federal Regulations, 5.11 & 5.15**

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WASHINGTON, D.C. 20231
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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
10/085,959	03/01/2002	1632	1038	960296.97648		11	5

CONFIRMATION NO. 4213

UPDATED FILING RECEIPT



OC000000008506281

Nicholas J. Seay
One South Pinckney Street, Suite 600
P.O. Box 2113
Madison, WI 53701-2113

Date Mailed: 07/24/2002

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Applicant(s)

Frederick R. Blattner, Madison, WI;
Rodney A. Welch, Madison, WI;
Valerie D. Burland, Cross Plains, WI;

Domestic Priority data as claimed by applicant**Foreign Applications****If Required, Foreign Filing License Granted 04/04/2002****Projected Publication Date: 09/04/2003****Non-Publication Request: No****Early Publication Request: No****Title**

Novel sequences of E. coli CFT073

Preliminary Class

435

**LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15**

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PTO/SB/05 (03-01)
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UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 CFR 1.53(b))		Attorney Docket No.	960296.97648
		First Inventor	Fredrick R. Blattner
		Title	NOVEL SEQUENCES OF E.COLI CFT073
		Express Mail Label No.	ET943407105US

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	ADDRESS TO: Assistant Commissioner for Patents Box Patent Application Washington, DC 20231
<p>1. <input checked="" type="checkbox"/> Fee Transmittal Form (e.g., PTO/SB/17) <i>(Submit an original and a duplicate for fee processing)</i></p> <p>2. <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.</p> <p>3. <input checked="" type="checkbox"/> Specification [Total Pages 765] <i>(Preferred arrangement set forth below)</i></p> <ul style="list-style-type: none">- Descriptive title of the invention- Cross Reference to Related Applications- Statement Regarding Fed sponsored R & D- Reference to sequence listing, a table, or a computer program listing appendix- Background of the Invention- Brief Summary of the Invention- Brief Description of the Drawings (if filed)- Detailed Description- Claim(s)- Abstract of the Disclosure <p>4. <input type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets <input type="text"/>]</p> <p>5. Oath or Declaration [Total Pages <input]<="" p="" type="text"/><p>a. <input checked="" type="checkbox"/> New UNSIGNED Copy from a prior application (37 CFR 1.63 (d))</p><p>b. <input type="checkbox"/> (for continuation/divisional with Box 18 completed)</p><p>i. <input type="checkbox"/> DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</p><p>6. <input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76</p></p>	<p>7. <input type="checkbox"/> CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix)</p> <p>8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)</p> <p>a. <input checked="" type="checkbox"/> Computer Readable Form (CRF)</p> <p>b. Specification Sequence Listing on:</p> <p>i. <input type="checkbox"/> CD-ROM or CD-R (2 copies); or</p> <p>ii. <input checked="" type="checkbox"/> paper</p> <p>c. <input checked="" type="checkbox"/> Statements verifying identity of above copies</p>
ACCOMPANYING APPLICATION PARTS	
<p>9. <input type="checkbox"/> Assignment Papers (cover sheet & document(s))</p> <p>10. <input type="checkbox"/> 37 CFR 3.73(b) Statement of Power of Attorney (when there is an assignee)</p> <p>11. <input type="checkbox"/> English Translation Document (if applicable)</p> <p>12. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449</p> <p>13. <input type="checkbox"/> Preliminary Amendment</p> <p>14. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) (Should be specifically itemized)</p> <p>15. <input type="checkbox"/> Certified Copy of Priority Document(s) (if foreign priority is claimed)</p> <p>16. <input type="checkbox"/> Nonpublication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.</p> <p>17. <input type="checkbox"/> Other: _____</p>	

18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP)


of prior application No.: _____ / _____

Prior application information:

Examiner _____

Group Art Unit: _____

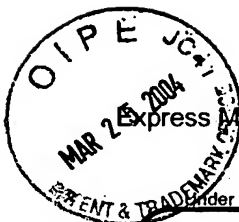
For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

19. CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number or Bar Code Label			or <input checked="" type="checkbox"/> Correspondence address below		
					
Name		Nicholas J. Seay			
Address		One South Pinckney Street, Suite 600			
City		Madison		State	WI
Country		US		Zip Code	53701-2113
Telephone		608-251-5000		Fax	608-251-9166

Name (Print/Type)	Bennett J. Berson	Registration No. (Attorney/Agent)	37,094
Signature		Date	October 19, 2001

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PTO/SB/17 (10-01)

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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FEE TRANSMITTAL for FY 2002

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT

(\$ 908.00)

Complete if Known

Application Number	
Filing Date	
First Named Inventor	Frederick R. Blattner
Examiner Name	
Group Art Unit	
Attorney Docket No.	960296.97648

METHOD OF PAYMENT

1. ☒ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit
Account
Number

17-0055

Deposit
Account
Name

Quarles & Brady LLP

Charge Any Additional Fee Required
Under 37 CFR 1.16 and 1.17Applicant claims small entity status.
See 37 CFR 1.27

2. **Payment Enclosed:**



Check



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FEE CALCULATION**1. BASIC FILING FEE**

Large Entity Small Entity

Fee Code (\$)	Fee Code (\$)	Fee Description
---------------	---------------	-----------------

101 740	201 370	Utility filing fee
106 330	206 165	Design filing fee
107 510	207 255	Plant filing fee
108 740	208 370	Reissue filing fee
114 160	214 80	Provisional filing fee

Fee Paid

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SUBTOTAL (1) (\$ 740.00)

2. EXTRA CLAIM FEES

Total Claims	Extra Claims	Fee from below	Fee Paid
11	-20** = 0	18.00	0.00
5	-3** = 2	84.00	168.00

Multiple Dependent

Large Entity Small Entity
Fee Code (\$)

Fee Code (\$)	Fee Code (\$)	Fee Description
103 18	203 9	Claims in excess of 20
102 84	202 42	Independent claims in excess of 3
104 280	204 140	Multiple dependent claim, if not paid
109 84	209 42	** Reissue independent claims over original patent
110 18	210 9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$ 168.00)

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FEE CALCULATION (continued)**3. ADDITIONAL FEES**

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
105 130	205 65	Surcharge - late filing fee or oath	
127 50	227 25	Surcharge - late provisional filing fee or cover sheet	
139 130	139 130	Non-English specification	
147 2,520	147 2,520	For filing a request for <i>ex parte</i> reexamination	
112 920*	112 920*	Requesting publication of SIR prior to Examiner action	
113 1,840*	113 1,840*	Requesting publication of SIR after Examiner action	
115 110	215 55	Extension for reply within first month	
116 400	216 200	Extension for reply within second month	
117 920	217 460	Extension for reply within third month	
118 1,440	218 720	Extension for reply within fourth month	
128 1,960	228 980	Extension for reply within fifth month	
119 320	219 160	Notice of Appeal	
120 320	220 160	Filing a brief in support of an appeal	
121 280	221 140	Request for oral hearing	
138 1,510	138 1,510	Petition to institute a public use proceeding	
140 110	240 55	Petition to revive - unavoidable	
141 1,280	241 640	Petition to revive - unintentional	
142 1,280	242 640	Utility issue fee (or reissue)	
143 460	243 230	Design issue fee	
144 620	244 310	Plant issue fee	
122 130	122 130	Petitions to the Commissioner	
123 50	123 50	Processing fee under 37 CFR 1.17(q)	
126 180	126 180	Submission of Information Disclosure Stmt	
581 40	581 40	Recording each patent assignment per property (times number of properties)	
146 740	246 370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149 740	249 370	For each additional invention to be examined (37 CFR § 1.129(b))	
179 740	279 370	Request for Continued Examination (RCE)	
169 900	169 900	Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$)

SUBMITTED BY

Name (Print/Type)

Bennett J. Berson

Registration No.
(Attorney/Agent)

37,094

Complete (if applicable)

Telephone

608-251-5000

Signature

Date

October 19, 2001

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NOVEL SEQUENCES OF *E. COLI* CFT073

CROSS-REFERENCE TO RELATED APPLICATION

[0001] Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with United States government support awarded by the following agency: NIH A144387. The United States has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] *Escherichia coli* is a common enteric bacterial strain that has both laboratory and human health importance. One particular strain of *E. coli*, designated CFT073 is a human pathogen that causes urinary tract infections. Urinary tract infections are common in various populations throughout the human life span. Infant boys, women of childbearing years, and aged people of both sexes have relatively high incidences of this infection. Acute pyelonephritis, a bacterial infection of the kidneys, is a common complication of such infections. Acute pyelonephritis often requires hospitalization for treatment, and the disease can be severe including complications or life threatening conditions.

[0004] Various strains of *E. coli* are associated with urinary tract infections and are commonly found in the urine of patients with pyelonephritis. Certain phenotypes of *E. coli* are found more often in such association. The strains associated with acute pyelonephritis often include a set of gene functions which, as a unit, have been thought to form a set of virulence factors that allow specific clones of *E. coli* to cause pyelonephritis.

[0005] Accordingly to investigate diagnosis or treatment of this disease, it is appropriate to focus the inquiry on the presumptive virulence factors. Most, if not all, of those virulence factors are present in the strain CFT073, which is known to be the among the most virulent of all of the *E. coli* strains associated with these diseases. The strain CFT073 has been previously shown to contain a pathogenicity island associated with uropathogenicity. Kao, JS et al., *Infect Immun.* 65:7, pp.2812-2920 (1997).

[0006] Modern geneticists have been working to resolve the genetic code of many organisms. Great efforts have been made to sequence the human genome. The effort to sequence the genomes of whole organisms began with an effort to sequence the genome of *E. coli*. For the original effort to sequence the *E. coli* genome, a useful and common laboratory strain, designated K-12, was chosen. The entire genome of that strain was sequenced and published. *Science*, 277:1453-1462 (1997). Since the genes which are responsible for the pathogenicity of *E. coli* CFT073 are missing from strain K-12, the sequence of the K-12 genome is of limited help in developing tools to detect, hinder or destroy *E. coli* CFT073.

BRIEF SUMMARY OF THE INVENTION

[0007] It is an object of the present invention to provide the DNA sequence present in *E. coli* CFT073 which is not present in non-pathogenic *E. coli* to enable detection, diagnosis, prophylaxis and therapeutic tools to combat bacterial infections.

[0008] It is another object of the present invention to provide a means to detect *E. coli* CFT073 in an infection of an environmental sample.

[0009] It is yet another object of this invention to provide a means for the early diagnosis of humans and livestock infected with CFT073.

[0010] Another object of the present invention is to provide a means of treating humans and livestock infected with CFT073.

[0011] It is a further object of the present invention to provide a means for the prevention of infection by CFT073.

[0012] The present invention includes many DNA sequences that are unique to *E. coli* CFT073.

[0013] One aspect of the present invention is two CFT073 DNA sequences that encode hemagglutinin-like proteins that are important for host cell adhesion.

[0014] Another aspect of the present invention is two CFT073 DNA sequences that encode for autotransporters.

[0015] Another aspect of the present invention is a CFT073 DNA sequence that encodes for a RTX-like protein.

[0016] Still another aspect of the present invention is a method for detecting *E. coli* CFT073 and distinguishing the strain from other strains of *E. coli* by genetic analysis and testing.

[0017] It is a feature of the invention disclosed here that virtually the entire genome of *E. coli* CFT073 is set forth in the data contained here, combined with the information already published in the field.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0018] Not applicable.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The investigators here have sequenced virtually the entire genome of *E. coli* CFT073. Presented in this specification is essentially all the DNA sequence which is contained in the genome of *E. coli* strain CFT073 and not found in the previously sequenced non-pathogenic laboratory *E. coli* strain K-12. The genome sequence is essentially complete, lacking only an occasional presumably small sequence linkage between established long sequences known. The availability of the sequence data presented here will enable intelligent design of diagnostic detection, prophylaxis and therapeutic tools for disease and infections caused by this organism.

[0020] The sequence of *E. coli* CFT073 was, in brief, performed by shotgun cloning and duplicative random sequence analysis followed by computer assembly into contigs. The contigs determined by shot gun clone sequencing were assembled using computer software designed for that purpose.

[0021] An important analysis which has begun on this sequence data is the identification of genetic sequences associated with the pathogenesis of infection, which sequences provide information essential to the diagnosis, treatment, and prevention of infection by uropathogenic *E. coli* strains. In order to facilitate the identification of genes involved in the pathogenesis of infection by uropathogenic *E. coli* strains for use in detection of the pathogen, and in the diagnosis, treatment, and prevention of uropathogenic infection, the entire genomic DNA sequence of *E. coli* CFT073 was compared with that of *E. coli* K-12, a nonpathogenic laboratory strain as published in *Science*, 277:1453-62 (1997).

[0022] Attached to this patent application is a sequence listing containing essentially all of the DNA sequence in the CFT073 genome, represented by the contigs mentioned above, that are not present in the K-12 genome. This sequence is present in the sequence listing as SEQ ID NO:1 through SEQ ID NO:251 and SEQ ID NO:254.

[0023] By definition, the genetic material in the sequences disclosed in this invention is sufficient for pathogenicity in humans since strain CFT073 is highly pathogenic while K-12 is not. In addition, analysis of the open reading frames (ORFs) and computer comparisons to sequences from other pathogens have allowed identification of several of the ORFs which code for proteins specifically associated with pathogenicity. We provide five examples of such ORFs. The first one (ORF1) is between nucleotide 12003 and nucleotide 20509 of SEQ ID NO:251. ORF1 is a putative member of the ShlA/HecA/Fha exoprotein family that shows a 25% identity over 2,311 residues to a probable hemagglutinin (the nucleotide sequence GenBank accession number for the probable hemagglutinin is AE004443). The second one (ORF2) is between nucleotide 31940 and nucleotide 34668 of SEQ ID NO:254. ORF2 is a member of the Shl/Fha/Hpm family and amino acids 33-907 of ORF2 shows a 26% identity to amino acids 1,912-2,818 of hemagglutinin/hemolysin-related protein (the amino acid sequence and the nucleotide sequence GenBank accession numbers are AAG03431 and AE002405, respectively). Both ORF1 and ORF2 are believed to be important for host cell adhesion and thus infection.

[0024] The third one (ORF3) is the complementary sequence between nucleotide 1008 and nucleotide 1885 of SEQ ID NO:85. ORF3 is a member of the autotransporter family and is 57% identical over 292 residues to a putative beta-barrel outer membrane protein (the nucleotide sequence GenBank accession number is AE005210). The fourth one (ORF4) is the complementary sequence between nucleotide 1996 and nucleotide 5607 of SEQ ID NO:85. ORF4 is also a member of the autotransporter family. ORF4 has a 31% identity over 1103 residues to YapD protein (the nucleotide sequence GenBank accession number is AJ277627) and a 27% identity over 2952 residues to YapH protein (the nucleotide sequence GenBank accession number is AJ277631). Both ORF3 and ORF4 as autotransporters are believed to be important virulence factors of CFT073.

[0025] The fifth one (ORF5) is between nucleotide 40329 and nucleotide 44950 of SEQ ID NO:251. ORF5 is similar to RTX family members and is 23% identical over 1,461 residues to a putative RTX family exoprotein (the nucleotide sequence GenBank accession number is AE005229). ORF5 is believed to be an exotoxin like RTX.

[0026] In addition to the diagnostic value, the DNA sequences of ORF1-5 are also useful for treatment and prevention purposes. For example, antisense oligonucleotides can be designed given the knowledge of these sequences to block the expression of the corresponding proteins. One of ordinary skill knows how to design and use antisense oligonucleotides. Along the

same line, the corresponding amino acid sequences of ORF1-5 or an immunogenic fragment thereof are also valuable for diagnosis, treatment and prevention of uropathogenic *E. coli* strains. For example, the corresponding amino acid sequences or an immunogenic fragment thereof can be used to generate antibodies that can be used for diagnosis, treatment and prevention purposes. One of ordinary skill in the art knows how to produce antibodies to the proteins encoded by ORF1-5. Vaccines may also be produced using the amino acid sequence information. It is well within the knowledge of a skilled artisan to generate vaccines.

[0027] The specific CFT073 strain from which the sequence data is derived is available from ATCC as ATCC 700928. One wishing to practice the present invention using one of the disclosed DNA sequences can do so by isolating the sequence from ATCC 700928 using knowledge of the nucleotide sequence and standard methods known to one of ordinary skill in the art.

[0028] It is expected that minor sequence variations in *E. coli* CFT073-specific nucleotide sequences associated with nucleotide additions, deletions, and mutations, whether naturally occurring or introduced *in vitro*, would not interfere with the usefulness of these sequences in the detection of uropathogenic *E. coli*, in methods for preventing urinary tract infection, and in methods for treating pyelonephritis. Therefore, the scope of the present invention is intended to encompass minor variations in the claimed sequences, which include both DNA and RNA and can also contain non-standard bases such as inosine.

[0029] Another utility enabled by the disclosure here is the detection of pathogenic *E. coli* strains by nucleic acid hybridization assays. Such assays, using techniques well known in the art, are made possible by the sequence information contained here, which enables the selection of CFT073-specific probes. By an *E. coli* CFT073-specific nucleotide probe, it is meant a sequence that is able to hybridize to *E. coli* CFT073 target DNA present in a sample containing *E. coli* CFT073 under suitable hybridization conditions and which does not hybridize with DNA from other *E. coli* strains or from other bacterial species. In particular, a CFT073 specific probe will bind to CFT073 DNA but not to DNA from K-12. This permits the intelligent design of DNA probes for use in hybridization assays for the presence of CFT073 strains. It is well within the ability of one skilled in the art to determine suitable hybridization conditions, based on probe length, G+C content, and the degree of stringency required for a particular application.

[0030] The probe may be RNA or DNA. Depending on the detection means employed, the probe may be unlabeled, radiolabeled, or labeled with a dye. The probe may be hybridized

with a sample that has been immobilized on a solid support such as nitrocellulose or a nylon membrane, or the probe may be immobilized on a solid support, such as a silicon chip.

[0031] The sample to be tested may include blood, urine, feces, or other materials from a human or a livestock animal. Alternatively, the sample may include food intended for human consumption. The sample may be tested directly, or may be treated in some manner prior to testing. For example, the sample may be subjected to PCR amplification using appropriate oligonucleotide primers.

[0032] Any means of detecting DNA-RNA or DNA-DNA hybridization known to the art may be used in the present invention.

[0033] Again, presented in this specification is a sequence listing constituting essentially all of the DNA sequence in the CFT073 genome that do not appear in strain K-12, which is presented as SEQ ID NO:1 to SEQ ID NO:251 and SEQ ID NO:254. Since all of these sequences are diagnostic of CFT073, as compared to K-12, sequence information from any of these sequences can be used to design diagnostic probes useful to distinguish strain CFT073 from strain K-12 using molecular techniques. To have reasonable assurance of success under conditions of variable stringency, it is preferred that such diagnostic probes use sequences which are at least 25 nucleotides or longer in length. Any 25-mer selected from amongst any of the sequences in any of SEQ ID NO:1 through SEQ ID NO:251 and SEQ ID NO:254 may be used for such a probe.

CLAIMS

WE CLAIM:

1. An isolated nucleic acid molecule comprising a nucleotide sequence or its complement, the nucleotide sequence is identical to at least twenty-five continuous nucleotides contained in DNA sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:251 and SEQ ID NO:254.
2. A recombinant nucleic acid construction comprising the isolated nucleic acid molecule of Claim 1.
3. A host cell comprising the recombinant nucleic acid construction of Claim 2.
4. A method for distinguishing *E. coli* bacteria of strain CFT073 from strain K-12 comprising analyzing the genome of the bacteria for the presence of any DNA sequence claimed in Claim 1.
5. A method for detecting *E. coli* CFT073 in a sample comprising the steps of:
 - a) providing an *E. coli* CFT073-specific nucleotide probe, the probe comprising a sequence or its complement, the sequence is selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:251 and SEQ ID NO:254;
 - b) contacting the probe with a sample of test material under suitable hybridization conditions; and
 - c) detecting hybridization of the probe to a complementary nucleotide sequence in the sample.
6. An isolated nucleic acid molecule comprising a nucleotide sequence or its complement, the nucleotide sequence is selected from the group consisting of the complementary sequence between nucleotide 1008 and nucleotide 1885 of SEQ ID NO:85, the complementary sequence between nucleotide 1996 and nucleotide 5607 of SEQ ID NO:85, the sequence between nucleotide 12003 and nucleotide 20509 of SEQ ID NO:251, the sequence between nucleotide 40329 and nucleotide 44950 of SEQ ID NO:251, and the sequence between nucleotide 31940 and nucleotide 34668 of SEQ ID NO:254.

7. A recombinant nucleic acid construction comprising the isolated nucleic acid molecule of Claim 6.

8. A host cell comprising the recombinant nucleic acid construction of Claim 7.

9. An isolated polypeptide comprising an amino acid sequence encoded by a DNA sequence selected from the group consisting of the complementary sequence between nucleotide 1008 and nucleotide 1885 of SEQ ID NO:85, the complementary sequence between nucleotide 1996 and nucleotide 5607 of SEQ ID NO:85, the sequence between nucleotide 12003 and nucleotide 20509 of SEQ ID NO:251, the sequence between nucleotide 40329 and nucleotide 44950 of SEQ ID NO:251, and the sequence between nucleotide 31940 and nucleotide 34668 of SEQ ID NO:254.

10. An isolated polypeptide comprising an immunogenic fragment of an amino acid sequence encoded by a DNA sequence selected from the group consisting of the complementary sequence between nucleotide 1008 and nucleotide 1885 of SEQ ID NO:85, the complementary sequence between nucleotide 1996 and nucleotide 5607 of SEQ ID NO:85, the sequence between nucleotide 12003 and nucleotide 20509 of SEQ ID NO:251, the sequence between nucleotide 40329 and nucleotide 44950 of SEQ ID NO:251, and the sequence between nucleotide 31940 and nucleotide 34668 of SEQ ID NO:254.

11. An antibody that specifically binds to the polypeptide of Claim 9.

ABSTRACT OF THE DISCLOSURE

The entire genome of pathogenic *E. coli* strain CFT073 has been sequenced. Nearly all of the genomic DNA sequences present in CFT073 and absent in the previously sequenced laboratory strain K-12 are presented here.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT

Applicants: Frederick R. Blattner
Rodney A. Welch
Valerie D. Burland

Date: October 19, 2001

Serial No.:

Art Unit:

Filed: Herewith

Examiner:

For: NOVEL SEQUENCES OF E.COLI CFT073

Docket: 960296.97648

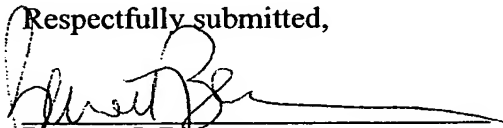
STATEMENT UNDER 37 C.F.R. 1.821(f)

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The undersigned, an attorney registered to practice before the U.S. Patent and Trademark Office and representing the applicants in the above-identified patent application does hereby state and affirm that the content of the printed computer listing contained within the above-identified patent application and the content of the sequence listing attached hereto in computer readable form are the same.

Respectfully submitted,



Bennett J. Berson

Reg. No. 37,094

Attorney for Applicants

QUARLES & BRADY LLP

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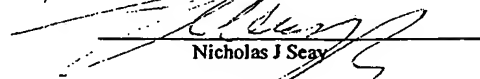


10/19/01	Chapter A-Ray 281677.900.18 75/943,579	Response
10/19/01	960296.97648 WARF Blattner, et al. ET 943407105US	Express Mail letter with Pat. App. Trans. Fee Transmittal (2) Application (6pg. Spec.) 2 pages of claims (11) 1pg abstract Opp. drawings 750 pages sequence listing
10/085959 3/81/02		Declaration + Power of Attorney Diskette containing Sequence Listing Statement under 37C.F.R. § 1.821G
10/19/01	960296.97648 WARF PCT Blattner, et al. ET 9434070916US PCT/us01/46833	PCT Int'l App. Trans. (1tr) PCT Request PCT Fee Calculation Description (6pg) Claims (3pg) Abstract (1pg) Drawings (none) Sequence Listing (786)
10/22/01	Warf 960296.96544 09/522,030	Fee Transmittal Supplemental IDS Form PTO-1449 w/attachments
10/24/01	Warf 960296.97290 PCT/us01/19638	Response to Invitation Power of Attorney Copy of Signature Authority Resolution



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Date of Signature and Deposit: July 5, 2002


Nicholas J Seay

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Frederick R. Blattner
Rodney A. Welch
Valerie D. Burland

Date: July 5, 2002

Serial No.: 10/085,959

Group Art Unit:

Filed: 03/01/2002

Examiner:

Title: NOVEL SEQUENCES OF E. COLI CFT073

File No.: 960296.97648

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FILED UNDER 37 CFR 1.53(b)
Filing Date Granted

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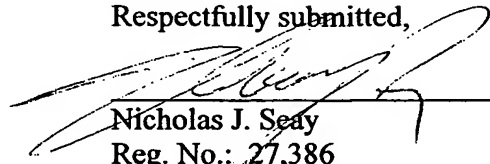
Dear Sir:

By a Notice mailed April 4, 2002 in the file of the above-identified application, the Patent and Trademark Office informed the applicants, through the undersigned, of the necessity of completing the filing of this application by filing a Declaration signed by the inventors. Enclosed herewith is a Declaration and Power of Attorney made out to the undersigned executed by all the inventors of this application. This Declaration was specifically attached to a complete copy of the specification and claims of this application at the time it was executed by the inventors and refers to the application by the same attorney docket number that makes specific reference to this patent application.

Wherefore it is believed that the filing particularities with regard to the filing of this application are completed. Examination on the merits in due course is respectfully requested.

A petition for extension of time for one month, plus the required surcharge is submitted herewith by a fee transmittal which accompanies this document.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Nicholas J. Seay", is written over a horizontal line.

Nicholas J. Seay
Reg. No.: 27,386
Attorney for Applicants
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QBMAD\335687.1

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Rev. 6/95U.S. Department of Commerce
Patent and Trademark Office

Attorney Docket Number

960296.97648

First Named Inventor

Frederick R. Blattner

**DECLARATION FOR
UTILITY OR DESIGN
PATENT APPLICATION****COMPLETE IF KNOWN**

Application Number

Filing Date

Group Art Unit

Examiner Name

☒ Declaration
Submitted with Initial Filing

OR

☐ Declaration
Submitted after
Initial Filing

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL SEQUENCES OF E. COLI CFT073

the specification of which

(Title of the Invention)

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY)

as United States Application Number or PCT International

Application Number

and was amended on (MM/DD/YYYY)

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
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☐ Additional foreign applications numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.
60/242,412	10/19/2000	

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DECLARATION

Page 2

I hereby claim benefit under Title 35, United States Code §120 of any United States application(s), or §365(C) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International application in the manner provided in the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and all continuation and divisional applications based thereon, and to transact all business in the Patent and Trademark Office connected therewith:

☐ Firm Name Customer or label Number
OR
☒ List attorney(s) and/or agent(s) name and registration number below

Name	Registration Number	Name	Registration Number
Herbert W. Mylius	24,578	Bennett J. Berson	37,094
Barry E. Sammons	25,608	Michael A. Jaskolski	37,551
Charles W. Jirauch	26,186	Richard T. Roche	38,599
Nicholas J. Seay	27,386	Terri S. Flynn	41,756
George E. Haas	27,642	John T. Pienkos	42,997
Michael J. McGovern	28,326	Daniel G. Radler	43,028
Carl R. Schwartz	29,437	Gregory M. Smith	43,136
Keith M. Baxter	31,233	Steven J. Wietrzny	44,402
John D. Franzini	31,356	Paul D. Amrozowicz	45,264
Janine R. Novatt	32,593	David M. Kettner	45,598
Jean C. Baker	35,433	Adam J. Forman	46,707
David G. Ryser	36,407	Zhibin Ren	47,897

☐ Additional attorney(s) and/or agents named on a supplemental priority sheet attached hereto

Please direct all correspondence to ☐ Customer Number or label OR ☒ Fill in correspondence address below

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Address: Quarles & Brady LLP
Address: P O Box 2113
City: Madison State: WI Zip: 53701-2113
Country: US Telephone: (608)251-5000 Fax: (608)251-9166

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given: Frederick Middle: R. Family: Blattner Suffix:

Inventor's Signature: *Frederick R Blattner* Date: Nov 27 2001

Residence: Madison State: WI Country: US Citizenship: US

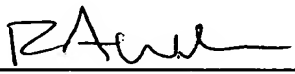
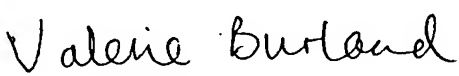
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☒ Additional inventors are being named on supplemental sheet(s) attached hereto

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DECLARATION										ADDITIONAL INVENTOR(S) Supplemental Sheet									
Name of Additional Joint Inventor, if any:										A petition has been filed for this unsigned inventor									
Given	Rodney				Middle	A		Family	Welch				Suffix						
Inventor's											Date	11/27/01							
Residence:	Madison				State	WI		Country	US				Citizenship	US					
Post Office	1825 Van Hise Avenue																		
Post Office																			
City	Madison				State	WI		Zip	53705		Country	US				Applicant Authority			
Name of Additional Joint Inventor, if any:										A petition has been filed for this unsigned inventor									
Given	Valerie				Middle Initial	D.		Family Name	Burland				Suffix						
Inventor's											Date	11-19-01							
Residence:	Cross Plains				State	WI		Country	US				Citizenship	British US VB 11-19-01					
Post Office	7966 Stagecoach Road																		
Post Office																			
City	Cross Plains				State	WI		Zip	53528		Country	US				Applicant Authority			
Name of Additional Joint Inventor, if any:										A petition has been filed for this unsigned inventor									
Given					Middle			Family					Suffix						
Inventor's											Date								
Residence:					State			Country					Citizenship						
Post Office																			
Post Office																			
City					State			Zip			Country					Applicant Authority			
Name of Additional Joint Inventor, if any:										A petition has been filed for this unsigned inventor									
Given					Middle			Family					Suffix						
Inventor's											Date								
Residence:					State			Country					Citizenship						
Post Office																			
Post Office																			
City					State			Zip			Country					Applicant Authority			
Name of Additional Joint Inventor, if any:										A petition has been filed for this unsigned inventor									
Given					Middle			Family					Suffix						
Inventor's											Date								
Residence:					State			Country					Citizenship						
Post Office																			
Post Office																			
City					State			Zip			Country					Applicant Authority			

☐ Additional inventors are being named on supplemental sheet(s) attached hereto